

Reduction of oxidative stress does not affect recovery of myocardial function: warm continuous versus cold intermittent blood cardioplegia

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Abstract

Objective—To compare oxidative stress after cardiac surgery in patients treated with two different methods of myocardial protection: warm continuous versus cold intermittent blood cardioplegia. To correlate oxidative stress with postoperative myocardial dysfunction.

Design—Prospective, randomised, double blind, trial.

Setting—Institutional centre of cardiovascular surgery.

Patients—20 patients were selected for coronary artery bypass surgery (CABG) on the following basis: stable angina, ejection fraction > 50%, double or triple vessel disease, no previous CABG or associated disease. Patients were randomised to two groups of 10 patients each.

Interventions—Patients underwent CABG with one of two different methods of myocardial protection and cardiopulmonary bypass. CBC group: intermittent cold blood antegrade-retrograde cardioplegia with moderate hypothermic cardiopulmonary bypass; WBC group: continuous warm blood antegrade-retrograde cardioplegia with mild hypothermic cardiopulmonary bypass.

Main outcome measure—The index of oxidative stress used was the alteration of whole blood and plasma glutathione redox status. Samples were collected from the coronary sinus and peripheral vein before anaesthesia (T1), before aortic unclamping (T2), 15 minutes (T3), and 30 minutes (T4) after unclamping. Haemodynamic parameters were measured with thermodilution techniques.

Results—Oxidised glutathione and glutathione-cysteine mixed disulphide significantly increased in the coronary sinus plasma in the CBC group, and the overall redox balance of glutathione was decreased ($P < 0.01$) at T2-T4 versus T1, and compared with the WBC group. Comparable results were obtained for coronary sinus blood. There was no correlation between postoperative haemodynamic measurements and oxidative stress markers.

Conclusions—Oxidative stress was significant in patients undergoing CABG using cold blood cardioplegia, while the warm technique minimised the effects of ischaemia. However, oxidative stress was not correlated with myocardial dysfunction following CABG.

(Heart 1997;77:465-473)

Keywords: oxygen free radicals; blood cardioplegia; coronary artery bypass graft

Knowledge of myocardial stunning as a primary consequence of reperfusion in humans remains elusive, compared with numerous in vitro perfused organ preparations and in vivo animal studies. Despite improvements in surgical and myocardial protection techniques, postoperative ventricular dysfunction after cardiac surgery is common clinically¹⁻³ and observed experimentally,⁴⁻⁶ and may represent "global stunning". In many cases there was no evidence of myocardial necrosis following total ischaemia by aortic clamping and reperfusion of the ventricle.⁷ Evidence of a role for oxygen derived free radicals is overwhelming in most experimental preparations: the generation of free radicals has been demonstrated and measured directly in myocardial tissue after a transient period of ischemia-reperfusion by spin trapping and electron paramagnetic resonance (EPR) techniques⁸⁻¹⁰; there is a relation between the activity of free radicals and the entity of stunning; antioxidant and free radical scavengers enhance recovery of function in stunned myocardium.¹¹⁻¹³ Confirmation of these findings in the postischaemic human myocardium has been elusive, as we must rely on indirect criteria to assess oxidative stress and the effects of anti-free radical interventions in the clinical setting.¹⁴⁻¹⁶ To date there is a limited number of reports suggesting the presence of oxidative stress following reperfusion of the postischaemic human heart.¹⁷⁻¹⁹ None of the studies prove that the magnitude of free radical formation found is harmful, although they do support the overall concept that free radicals are formed during reperfusion and may contribute to reperfusion injury.^{20, 21} Nonetheless, Ferrari *et al* have reported the occurrence of major changes of glutathione content and redox balance in coronary sinus plasma during postischaemic reperfusion in patients subjected to crystalloid cardioplegia for coronary artery bypass grafting (CABG).¹⁷ They found a positive correlation between the duration of ischaemia and the release of oxidised glutathione; in addition, the recovery of the cardiac index was inversely related to the measurements of oxidative stress. Furthermore, Bolli *et al* have clarified that the degree of postischaemic dysfunction is primarily determined by the severity and duration of previous ischaemia.⁸ Using EPR, they

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Accepted for publication
19 February 1997

showed a linear relation between the magnitude of free radical generation and ischaemic flow reduction. These findings imply that, regardless of the precise mechanism responsible for stunning, the mechanism must be initiated and regulated by ischaemia, and any intervention that improves perfusion during ischaemia would be expected to attenuate stunning after reperfusion.

These findings prompted our investigation of a new method of myocardial protection developed by Lichtenstein *et al.*²² The basic concept of this method is the association of chemical electromechanical arrest (by potassium) with a continuous warm blood (WBC) perfusion to prevent myocardial ischaemia. In the past few years, methods have been developed to modify cardioplegia and reperfusion constituents, or to supply various antioxidants with the aim of reducing reperfusion injury after cardioplegic arrest during cardiac surgery.²³ The goal of WBC is not only to provide substrate, oxygen, and perfusion to achieve some degree of cellular repair but also to prevent additional damage from ischaemia, hypothermia, reperfusion, and oxidative stress.

In the present study, the alterations of glutathione redox status (taken as an indirect index of oxidative stress) occurring in plasma and whole blood were measured into two groups of 10 patients undergoing different techniques of myocardial protection for CABG. Furthermore, the presence of a correlation between oxidative stress and myocardial function in the early postoperative hours was investigated.

Patients and methods

PATIENTS

From January to July 1993, 20 patients scheduled for elective CABG were selected on the basis of symptoms and cardiac catheterisation—stable chronic angina, double or triple coronary artery vessels disease (> 50% reduction in luminal diameter), and preserved left ventricular function (ejection fraction > 50%, left ventricular end diastolic pressure < 15 mm Hg, no dyskinetic alteration of left ventricular wall motion). To obtain homogeneous groups we excluded patients with recent myocardial infarction (within one year), previous CABG, combined surgical procedures, pharmacologically treated diabetes, severe hypertension (systolic blood pressure > 180 mm Hg, diastolic blood pressure > 120 mm Hg), and associated diseases.

The surgical staff was the same for all the patients. The extent of coronary artery disease and general patient characteristics are shown in table 1. The 20 patients were randomised into two groups. Each patient scheduled for surgery was assigned a progressive number; patients with odd numbers were randomised to the WBC technique, and patients with even numbers to intermittent cold blood cardioplegia (CBC). All patients gave informed consent to participate in this study, which had the approval of the local medical ethics committee. Patients treated with allopurinol or other

antioxidant agents were excluded from the present study.

SURGICAL TECHNIQUE

Antianginal medications (nitrates and calcium channel blockers) were continued until the morning of operation. Anaesthesia was induced with fentanyl (5 mg/kg) combined with diazepam (0.3 mg/kg) and maintained with fentanyl infusion (25 µg/kg/min). Muscle relaxation was achieved with pancuronium bromide (0.1 mg/kg). Ventilation with a mixture of oxygen and nitrous oxide (50:50) was adjusted to maintain normocapnia. In the CBC group, during bypass haematocrit was maintained between 20% and 25% and pump flows were maintained between 2.0 and 2.2 l/min/m². In the WBC group, to supply the increased metabolic requirements, flow rates were between 2.2 and 2.5 l/min/m² and haematocrit between 22% and 25%. In both groups, mean arterial pressure was maintained between 60 and 70 mm Hg using sodium nitroprusside (0.5–5 µg/kg/min) or noradrenaline (0.01–0.5 µg/kg/min).

In both groups coronary sinus was cannulated blindly with a Research Medical Inc retroplegic cannula (Salt Lake City, Utah, USA); venous drainage was by a two stage cannula and internal mammary artery bypasses were implanted as the last anastomoses.

CARDIOPLEGIC TECHNIQUES

CBC group

Cold blood cardioplegia was administered at a 4:1 ratio of oxygenated circuit blood and crystalloid solution. The physical and biochemical composition of the blood cardioplegic solution were in accordance with the protocol suggested by the UCLA Medical Centre²⁴ except that our solution did not contain aspartate and glutamate. The delivery was sequentially divided between antegrade and retrograde through the aorta and through the coronary sinus; arrest was achieved with an initial cold (4–8°C) blood “high potassium” solution (20–25 mEq/l) with a flow rate of 200–300 ml/min for two minutes through the aorta and then for two minutes through the coronary sinus where the perfusion pressure was maintained below 40 mm Hg. On completion of each distal-proximal anastomoses or at 20 minute intervals, potassium solution (8–10 mEq/l) was administered at a flow rate of 150–200 ml/min for one minute through the aorta and then for one minute through the coronary sinus. Before cross clamp release, warm (37°C) blood “low potassium” solution was infused at a flow rate of 150 ml/min, alternating between the aortic root and the coronary sinus at a pressure of 50 mm Hg for three to five minutes.^{23 24} In this group moderate systemic hypothermia (28–30°C) and topical hypothermia were used.

WBC group

Warm blood perfusion was administered through the combined antegrade (aorta) and retrograde (coronary sinus) route. Oxygenated blood was taken from the arterial line of the

Table 1 Clinical information

	CBC group (n = 10)	WBC group (n = 10)
Age (years)	60.7 (10.1)	62.3 (11.1)
Sex (male/female)	9/1	9/1
CCS class (preop)	2.75 (0.4)	2.9 (0.6)
Diseased vessels (No/patient)	2.4 (0.8)	2.6 (0.5)
LV class (AHA)	1.3 (0.4)	1.6 (0.5)
MI (preop)	6	7
Grafts (No/patient)	2.75 (0.9)	3 (0.8)
CPB time (min)	123 (36)	109 (43)
Cross clamp time (min)	93 (33)	90 (23)
CK max (U/l)	1126 (695)	1850 (1357)
CK-MB max (U/L)	68 (23)	65 (19)

Values and mean (SD). P not significant for all variable by student Newman-Kewels *t* test. CCS class, Canadian Cardiovascular Society angina class; LV class (AHA), left ventricular class (American Heart Association); MI, myocardial infarction; CBP, cardiopulmonary bypass; CK, creatine kinase.

bypass circuit and delivered with a roller pump, potassium was added with a syringe pump (16 mEq of K⁺ and 3 mEq of Mg⁺⁺ per 20 ml) directly into a side branch of the cardioplegic line. In order to induce arrest, the first 20 ml of hyperkalaemic blood were infused in 30–60 seconds, the heart was then perfused antegradely for two minutes at a flow rate of 250–300 ml/min and then for two minutes retrogradely at 150–200 ml/min (retrograde pressure < 40 mm Hg). Subsequently, continuous perfusion was maintained with a retrograde perfusion flow rate of 150 ml/min,

and coronary sinus pressure < 40 mm Hg. Potassium infusion was regulated according to the Fabri formula²⁵ to obtain a K⁺ serum concentration of about 5.5 mEq/l. In this group systemic mild hypothermia (34–35°C) was obtained by allowing for a drift of core temperature at room temperature. The use of “blower” devices was requested to optimise the visualisation of the anastomoses procedures.

DATA ACQUISITION AND BIOCHEMICAL ASSAY
Sampling of plasma and whole blood for measurements of reduced glutathione (GSH), oxidised glutathione (GSSG), and glutathione-cysteine mixed disulphide (Cys-SG) was done in the operating theatre; samples included coronary venous blood from the retrograde cannula, and peripheral blood from venous return of the cardiopulmonary circuit. Blood samples for control measurements were obtained from peripheral veins of healthy subjects. The measurements were made by high performance liquid chromatography (HPLC) according to Reed *et al.*²⁶ Each blood sample was divided in two 100 µl aliquots: one was immediately dropped in 0.9 ml of 0.6% perchloric acid, mixed, and stored at –80°C; the other was centrifuged, the plasma fraction

Figure 1 Modifications of the glutathione redox status in the coronary sinus plasma during cardioplegic arrest and reperfusion. Data are reported as median, 25th, and 75th centile. T1, Before cardioplegia and the start of cardiopulmonary bypass; T2, one minute before aortic unclamping; T3, 15 minutes after aortic unclamping and reperfusion; T4, 30 minutes after aortic unclamping reperfusion. GSH, reduced glutathione; GSSG, oxidised glutathione; Cys-SG, glutathione-cysteine mixed disulphide; Ratio, GSH/(GSSG + Cys-SG). CBC group, intermittent blood cardioplegia and warm modified reperfusion; WBC group, continuous warm blood cardioplegia. *CBC values significantly different from control (*P* < 0.05); †CBC values significantly different from T1 (*P* < 0.05); ‡CBC values significantly different from WBC (*P* < 0.03); §CBC values significantly different from WBC (*P* < 0.01).

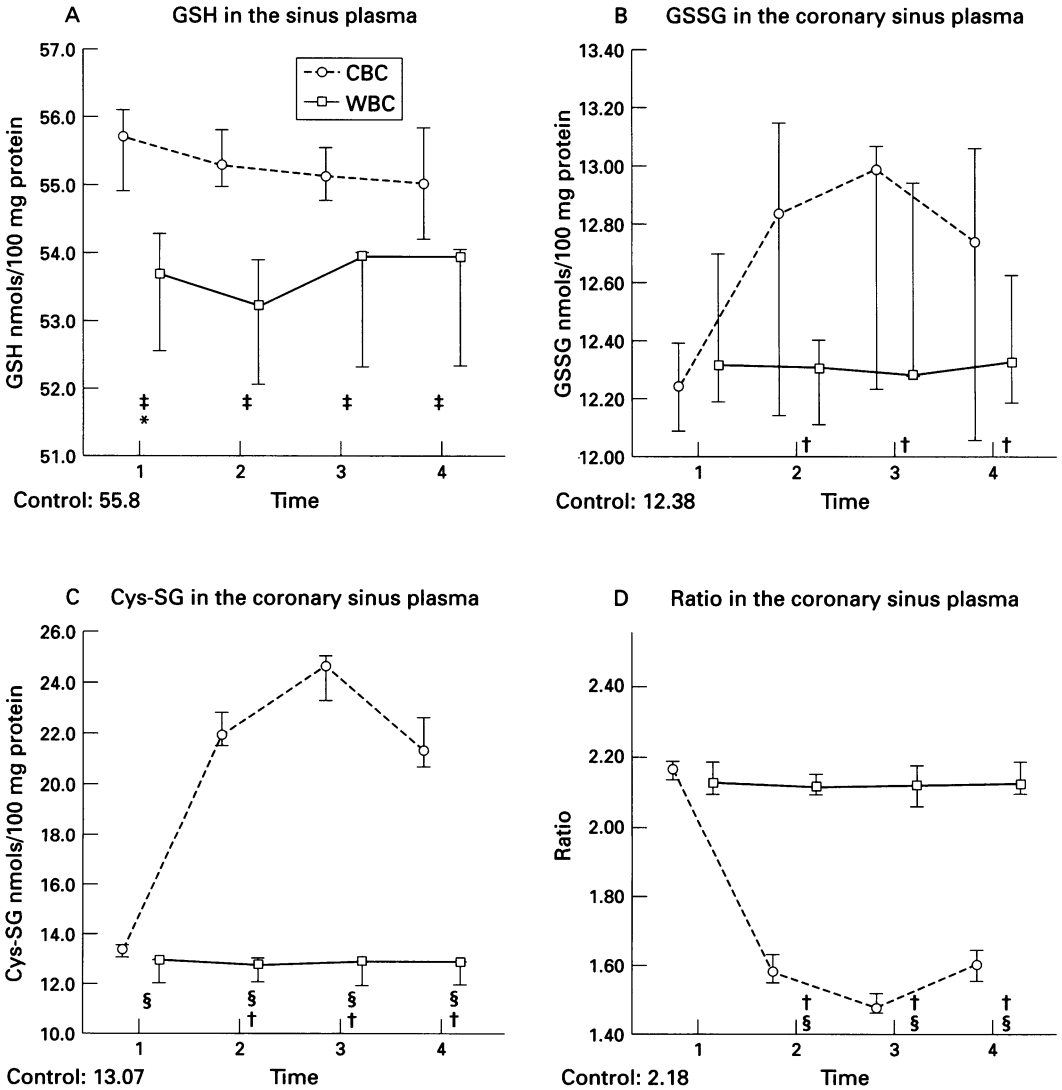
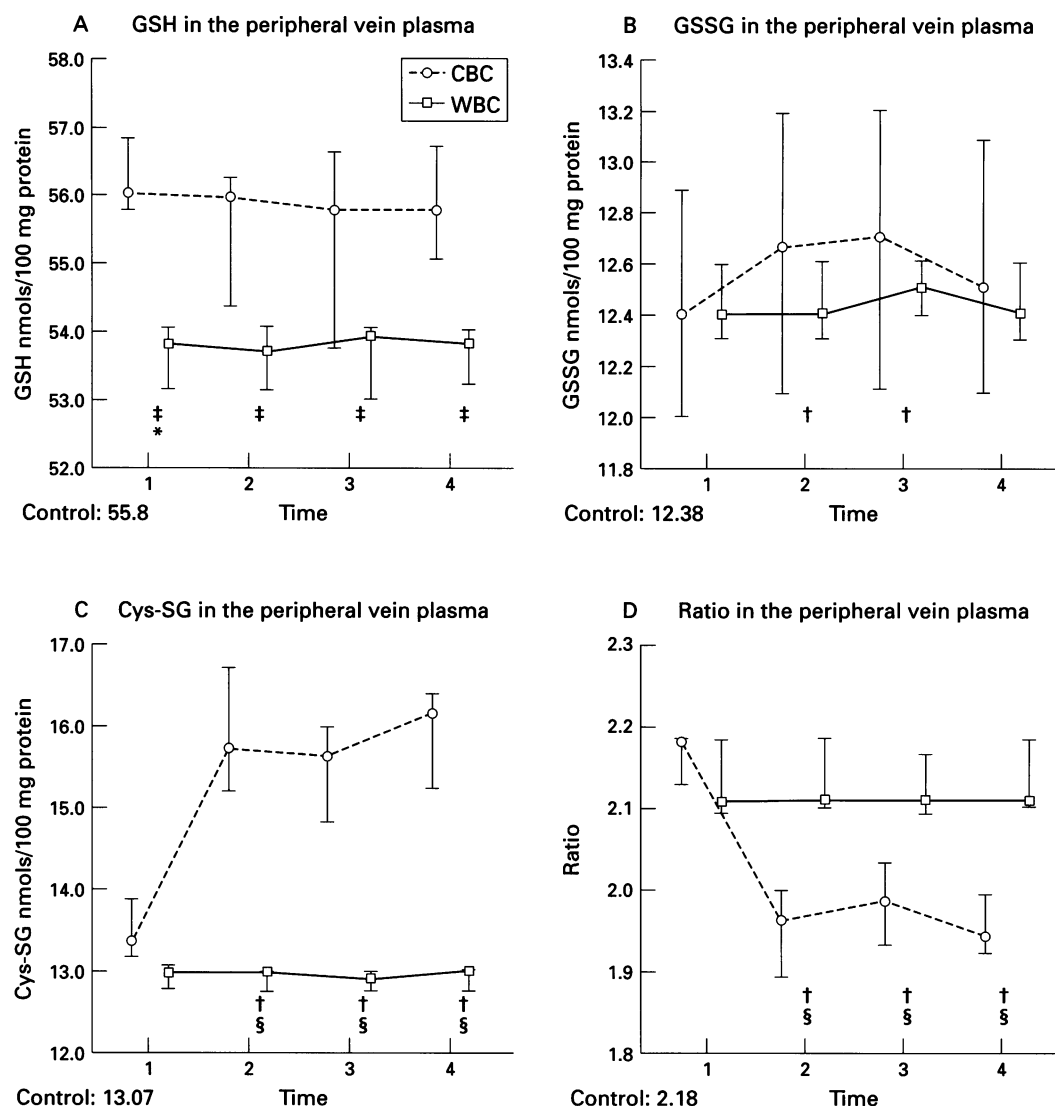


Figure 2 Modifications of the glutathione redox status in the peripheral vein plasma during cardioplegic arrest and reperfusion. Data are reported as median, 25th, and 75th centile. T1, Before cardioplegia and the start of cardiopulmonary bypass; T2, one minute before aortic unclamping; T3, 15 minutes after aortic unclamping and reperfusion; T4, 30 minutes after aortic unclamping and reperfusion. GSH, reduced glutathione; GSSG, oxidised glutathione; Cys-SG, glutathione-cysteine mixed disulphide; Ratio, $GSH/(GSSG + Cys-SG)$. CBC group, intermittent blood cardioplegia and warm modified reperfusion; WBC group, continuous warm blood cardioplegia. *CBC values significantly different from control ($P < 0.05$); †CBC values significantly different from T1 ($P < 0.05$); ‡CBC values significantly different from WBC ($P < 0.03$); §CBC values significantly different from WBC ($P < 0.01$).



removed and divided into aliquots, and stored at -80°C . Before GSH measurements, 0.5 ml of plasma were energetically mixed with 50 ml of 60% perchloric acid. The plasma and the whole blood precipitates were removed by high speed centrifugation ($17\,000 \times g$ for 10 minutes) and the supernatant used for GSH assay. The samples were analysed by HPLC using an NH2 m-Bondpak column, a step gradient of methanol-ammonium acetate-acetic acid and spectrophotometric detection at 365 nm. GSH, GSSG, and Cys-SG were measured by identifying the corresponding peaks which were compared with a calibration curve obtained with pure standards. Concentrations of plasma GSH, GSSG, and Cys-SG were expressed as nmol/100 mg protein.

Sampling times were: before cardioplegia and before the start of cardiopulmonary bypass (T1); one minute before aortic unclamping (T2); 15 minutes after aortic unclamping and reperfusion (T3); and 30 minutes after aortic unclamping and reperfusion (T4).

HAEMODYNAMIC AND METABOLIC MEASUREMENTS

Haemodynamic and metabolic measurements, by thermodilution technique, were: heart rate,

left atrial pressure (LAP), mean systemic arterial pressure (MAP), and cardiac output. Cardiac index (CI), left ventricular stroke work index (LVSWI), systemic vascular resistance (SVR), and O_2 delivery were determined by standard formulas. From LVSWI and LAP, we calculated the ventricular function (VF), according to the following equation:

$$VF = 11 \text{ artg} (\log LVSW - 1) / \log LAP$$

where artg is the trigonometric function arc tangent. VF is an index of left ventricular performance that describes the ventricular stroke work as a function of filling pressure.²⁷

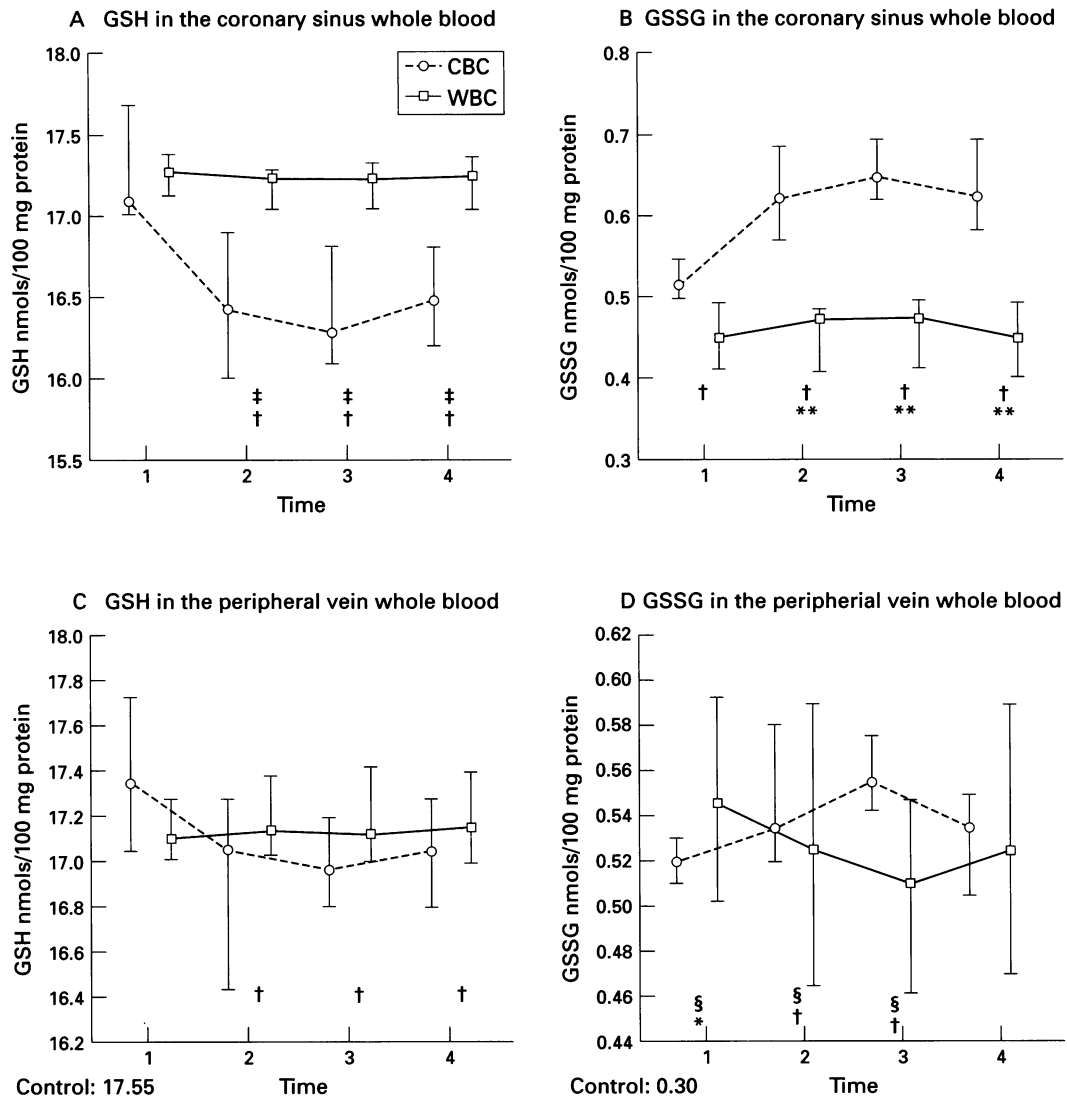
Arterial and mixed venous blood were analysed immediately at 37°C for blood gases and pH (ABL4, Radiometer, Copenhagen, Denmark). In particular, partial oxygen pressure in mixed venous blood (PVO_2) and arteriovenous oxygen difference (avO_2D) were measured. Haemoglobin and oxygen saturation were measured directly (IL 482 Co-Oximeter System, Instrumental Laboratory, Lexington, Massachusetts, USA). Oxygen content was calculated from the formula:

$$\text{O}_2 \text{ content} = 1.36 \text{ Hb} \cdot \text{S} + 0.0031 \text{ PO}_2$$

where PO_2 is the partial oxygen pressure.

A carbon dioxide analyser (930 Siemens

Figure 3 Modifications of the glutathione redox status in whole blood from the coronary sinus and peripheral vein during cardioplegic arrest and reperfusion. Data are reported as median, 25th, and 75th centile. T1, Before cardioplegia and the start of cardiopulmonary bypass; T2, one minute before aortic unclamping; T3, 15 minutes after aortic unclamping and reperfusion; T4, 30 minutes after aortic unclamping reperfusion. GSH, reduced glutathione; GSSG, oxidised glutathione; Cys-SG, glutathione-cysteine mixed disulphide; Ratio, GSH/(GSSG + Cys-SG). CBC group, intermittent blood cardioplegia and warm modified reperfusion; WBC group, continuous warm blood cardioplegia. *CBC values significantly different from control ($P < 0.05$); †CBC values significantly different from T1 ($P < 0.05$); ‡CBC values significantly different from WBC ($P < 0.03$); §CBC values significantly different from WBC ($P < 0.002$); **CBC values significantly different from WBC ($P < 0.01$).



Elema, Sweden) connected to the ventilator (900 C, Siemens Elema) was used to record continuously end-tidal carbon dioxide and minute carbon dioxide production index (VCO_2I). Haemodynamic and metabolic data were collected from a total of 100 patients (including the two study groups) for each cardioplegic technique, selected, and randomised according to the aforementioned criteria. Blood specimens were also obtained to analyse cardiac enzymes: total serum activities of creatine kinase were determined by an enzymatic method and the activity of creatine kinase MB isoenzyme was measured by immunoassay. Measurements were recorded: before induction of anaesthesia (T1), before sternotomy (T2), at admission to the intensive care unit (T3), and at 3, 6, 9, and 24 hours after ICU admission (T4, T5, T6, T7). After discontinuation of cardiopulmonary bypass, measurements were obtained when the patients were haemodynamically stable.

STATISTICAL ANALYSIS

The median and the 25th and 75th centile of each variable of the glutathione redox status was determined, the median values compared using the rank Mann-Whitney test. The mean (SD) of haemodynamic parameters were sup-

plied and a modified t test (Student-Newman-Keuls) and χ^2 test for the two groups was applied. ANOVA linear regression analysis was applied to correlate enzymes versus Cys-SG measurements, and cross clamping time versus glutathione redox balance.

Results

The groups (table 1) overlapped in respect to vessel disease and left ventricular preoperative class, even the expected difference in cardiopulmonary bypass time for different rewarming times was not significant. Minor differences were observed in creatine kinase and MB isoenzyme concentrations.

The modifications of glutathione redox status in the coronary sinus and peripheral vein plasma of patients undergoing the two different methods of myocardial protection are shown in figures 1 and 2.

Neither group had a significant decrease of coronary sinus plasma GSH from T1 to T4. In the CBC group, GSSG and Cys-SG concentrations increased and remained sustained for at least for 30 minutes after reperfusion, and the overall redox balance of glutathione decreased markedly. The difference was statistically significant for GSSG at T2, T3, and T4

Table 2 Changes in physiological variables after cardiopulmonary bypass

	T1	T2	T3	T4	T5	T6	T7
CBC group							
Hb (g/100ml)	12.9 (1.3)	13.0 (1.3)	9.3 (1.5)	9.6 (1.2)	8.9 (1.5)	8.9 (1.3)	9.6 (0.9)
avO ₂ D (vol %)	3.4 (0.6)	4.8 (1.1)	4.6 (0.8)	5.2 (0.9)	4.4 (0.7)	4.0 (0.5)*	4.3 (0.8)
HR (beats/min)	71.3 (17.3)	71.3 (10.6)	94.5 (16.9)	106 (14.6)	106 (19.3)	106 (15.0)	100 (9.1)
LAP (mmHg)	10.2 (2.6)	10.0 (3.1)	9.2 (3.7)	11.1 (2.6)	11.4 (3.2)	10.6 (2.2)	10.4 (3.4)
SVR (dyn.s.cm ⁻⁵)	1291 (432)	1559 (264)	1790 (472)	1075 (293)	1017 (233)	1015 (271)	1310 (275)*
VCO ₂ I (ml min/m ²)		280 (39)	192 (45)†	306 (85)	287 (55)	270 (51)	
CI (l min/m ²)	3.3 (1.1)	2.2 (0.3)	2.2 (0.6)	2.9 (0.6)	3 (0.5)	3.1 (0.5)	2.9 (0.5)
LVSWI (g m/m ²)	60.4 (11.7)	35.5 (5.3)	30.8 (10)	30.4 (8.8)	30.5 (6.6)	31.2 (5.0)	37 (10.0)
VF (rad)	8.8 (0.9)	7.6 (0.9)	7.5 (2.4)	6.7 (1.4)	6.8 (1.2)	7 (1.0)	7.4 (1.1)
DO ₂ I (ml min/m ²)	304 (110)	222 (30)	158 (35)†	213 (42)	209 (39)	212 (27)	208 (46)
WBC group							
Hb (g/100ml)	12.8 (1.1)	12.6 (0.8)	10.1 (1.2)	9.9 (1.2)	9.4 (1.3)	9.1 (1.3)	9.4 (1.4)
avO ₂ D (vol %)	3.4 (0.7)	4.7 (0.6)	4.9 (0.9)	4.8 (0.9)	4.4 (0.9)	4.6 (1.0)	3.9 (0.6)
HR (b/min)	68.2 (9.5)	66.8 (8.5)	104 (18.5)	101 (12.8)	95.3 (8.8)	91.4 (10.6)	89.6 (7.3)
LAP (mmHg)	10.2 (4.7)	10.3 (4.0)	10.1 (2.8)	10.1 (2.8)	10.8 (2.4)	9.9 (2.7)	11.2 (2.4)
SVR (dyn.s.cm ⁻⁵)	1297 (427)	1555 (525)	1521 (455)	1155 (364)	1120 (349)	1016 (263)	986 (394)
VCO ₂ I (ml min/m ²)		231 (43)	234 (37)	291 (60)	293 (53)	281 (58)	
CI (l min/m ²)	3.2 (0.8)	2.1 (0.4)	2.6 (0.7)	2.7 (0.5)	2.9 (0.7)	3.0 (0.4)	3.3 (0.6)
LVSWI (g m/m ²)	63 (17.7)	32.6 (7.5)	33.4 (9.1)	29.4 (4.2)	32.7 (9.5)	34.0 (5.8)	39.5 (7.4)
VF (rad)	9.1 (0.8)	7.3 (1.2)	7.3 (0.6)	6.9 (0.6)	7 (0.7)	7.5 (0.6)	7.5 (0.6)
DO ₂ I (ml min/m ²)	255 (54)	182 (38)	201 (60)	213 (75)	209 (70)	208 (48)	223 (45)

Values are reported as mean (SD). *Significantly different (P < 0.03) from the WBC group; †Significantly different (P < 0.01) from the WBC group. T1, before anaesthesia induction; T2 before sternotomy; T3, at the admission in the intensive care unit; T4–T7, at 3, 6, 9, 24 hours later. CBC group, intermittent blood cardioplegia and warm modified reperfusion; WBC group, continuous warm blood cardioplegia. Hb, haemoglobin; avO₂D, arteriovenous oxygen difference; HR, heart rate; LAP, left atrial pressure; SVR, systemic vascular resistances; VCO₂I, minute carbon dioxide production index; CI, cardiac index; LVSWI, left ventricular stroke work index; VF, ventricular function; DO₂I, oxygen delivery index.

versus T1, for CyS-SG and for the ratio (GSH/(GSSG + Cys-SG)) at T2, T3, and T4 versus T1 and compared with the WBC group in which glutathione redox status was almost unmodified (fig 1). In addition, only the CBC group showed similar but not so substantial modifications of the same variables in parallel measurements performed on plasma obtained from a peripheral vein (fig 2).

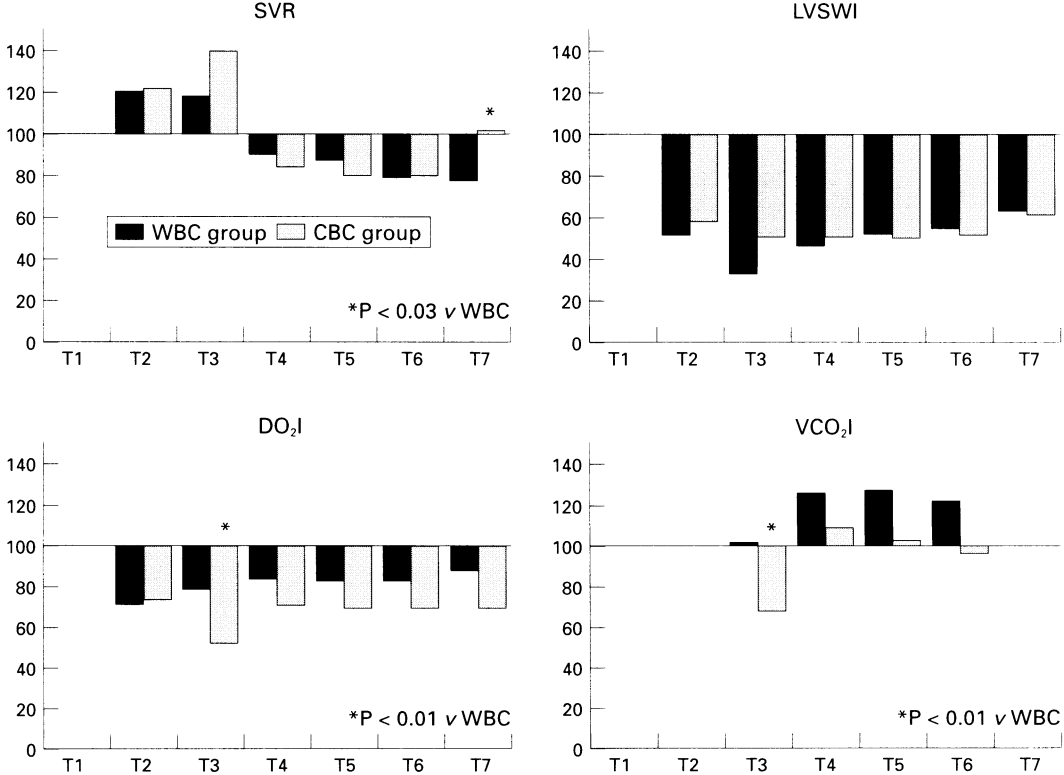
However, none of these parameters in either group was significantly correlated with the duration of cardioplegic arrest (cross clamping time) or the cardiospecific enzymes (creatine kinase MB isoenzyme) (correlation coefficient < 0.40, P > 0.5).

The modifications of glutathione redox status in whole blood (erythrocytes) are shown in

figure 3. Heart reperfusion after cardioplegia was associated with a moderate decrease of GSH and a concomitant increase of GSSG in the coronary sinus whole blood of the CBC group. These differences were significant for T1 versus T2, T3, and T4, and compared with the WBC group at same times. Only minor variations were observed in the peripheral venous blood of the CBC group, while no significant differences were measured in the coronary sinus and peripheral venous whole blood of the WBC group at any time.

Table 2 and figure 4 show the haemodynamic changes after cardiopulmonary bypass for the two groups. Recovery progressively improved during the six hours after ICU admission. LVSWI remained lower than pre-

Figure 4 Most significant variables as a percentage of basal time at each observation time in the two groups. T1, before anaesthesia induction; T2, before sternotomy; T3, at admission to intensive care unit; T4–T7, 3, 6, 9, and 24 hours later. CBC group, intermittent blood cardioplegia and warm modified reperfusion; WBC group, continuous warm blood cardioplegia; SVR, systemic vascular resistance; LVSWI, left ventricular stroke work index; DO₂I, oxygen delivery index; VCO₂I, minute carbon dioxide production index.



bypass values while CI, LAP, SVR, O₂ delivery and avO₂D values initially indicated good cardiovascular performance without statistically significant differences between the two groups, especially at 24 hours after CABG.

Discussion

Our knowledge of myocardial stunning and the involvement of free radicals in postischaemic injury in human heart has been relatively elusive compared with the amount of experimental data.^{15 17 19} Whether reperfusion injury is clinically relevant in patients undergoing myocardial reperfusion after global ischaemia during cardioplegic arrest (cardiac surgery), or after thrombolytic or angioplasty therapy for myocardial infarction remains controversial.²⁸⁻³⁰ Postoperative acute myocardial dysfunction has been suggested to be a common occurrence after cardiopulmonary bypass.¹⁻³ It appears to be a reversible event within 24 hours of surgery and may have importance in the early postoperative period, especially in patients whose ventricular function is already compromised. Some studies have suggested that inadequate myocardial protection or cold cardioplegia^{31 32} together with reperfusion injury from oxygen derived free radicals are factors in postoperative myocardial dysfunction.^{33 34} Because reperfusion is a prerequisite for the survival of ischaemic tissue, the proof of the existence of reperfusion induced injury essentially relies on the demonstration of limitation of myocardial injury by interventions that are implemented at the time of reperfusion. However, we are waiting confirmation that stunning indicates injury at the time of reperfusion rather than being a sign of delayed recovery after ischaemia.^{13 35-37}

The method of myocardial protection developed by Lichtenstein *et al*²² that involves chemical electromechanical arrest and continuous warm blood perfusion aims to prevent damage from ischaemia, hypothermia, reperfusion, and oxidative stress.

As reported by Sies³⁸ oxidative stress can be defined as a condition in which the balance between pro-oxidants and antioxidants is shifted in favour of the former: among the various intracellular antioxidants an important role is played by GSH that acts as a reductant in enzymatic reactions catalysed by peroxidase and thiol disulphide oxidoreductases.^{39 40} Glutathione exists in three forms: GSH (reduced), GSSG (oxidised), and Cys-SG (glutathione mixed disulphides with low molecular weight thiols such as cysteine) or with thiol residues in protein.⁴¹ Intracellular glutathione concentrations, kept in the millimolar range by continuous synthesis, and the redox ratio between GSH, and GSSG and Cys-SG are usually extremely high. The redox balance is different in extracellular fluids (plasma) where the concentrations of GSSG and mixed disulphides are relatively high. However, following oxidative stress, GSH is enzymatically oxidised to GSSG which, in turn, may be re-reduced or actively excreted from the cell.⁴²

For these reasons an increased concentration of GSSG and a decrease of the glutathione redox balance in intracellular as well as extracellular fluids have been proposed as suitable indexes of oxidative stress.⁴³

The present study intended to demonstrate that postcardioplegia free radical production was detectable in patients undergoing cardiac surgery and that reduction (or suppression) of oxidative stress could be achieved by avoiding or limiting ischaemia and consequently reducing reperfusion injury without supplying antioxidants or modifications of constituents of cardioplegia as used in recent years.²³

The results from the CBC group show that during reperfusion of ischaemic human hearts the plasma concentrations of the oxidised form of glutathione increased in the coronary sinus, indicating oxidative stress, while the WBC group did not show any important alterations in the glutathione redox status, suggesting that this method minimises the effects of ischaemia and prevents additional damage from hypothermia and reperfusion.

A moderate but significant decrease in the basal concentration of GSH was found in the WBC group in both coronary sinus and peripheral vein plasma compared with control subjects (figs 1 and 2). As the decrease was not paralleled by a concomitant increase of the oxidised forms of glutathione, one can argue that this was a decreased supply rather than oxidation.

It has been claimed^{19-27 38-41 44} that erythrocytes are a natural circulating reservoir of antioxidants to be employed during oxidative stress in various organs and tissues. A change in erythrocyte glutathione redox status during reperfusion would therefore reflect both the occurrence of an oxidative stress and the involvement of erythrocyte glutathione as an antioxidant defence mechanism. More than 98% of total blood glutathione is in the erythrocytes, therefore, a simple measure of total blood glutathione reflects the concentration of glutathione in erythrocytes.

Plasma glutathione measurements are mirrored in whole blood from either the coronary sinus or the peripheral vein, showing an increase of oxidised forms only in the CBC group (fig 3). Furthermore, a significant increase of GSSG was detected in whole blood (coronary sinus and peripheral vein) from both groups before cardioplegic arrest compared with controls (fig 3B). This difference could be related to the induction of anaesthesia, and aortic and caval cannulation as a global "stress"; however, during reperfusion the concentration of GSSG markedly increased in the CBC group especially in whole blood from the coronary sinus.

Signs of oxidative stress were also found in peripheral blood from the CBC group and this may reflect either a lesser increase of oxidised forms in the peripheral vein secondary to an initial greater generation at the myocardium, or the involvement of other tissues in the oxidative process related to general activation of polymorphonuclear leucocytes by cardiopulmonary bypass. The first hypothesis is

more likely because this increase in oxidised forms was not detected in the peripheral blood of the WBC group that underwent similar cardiopulmonary bypass.

Another criticism could be related to the difference in the delivery technique: intermittent versus continuous. Washout is usually a matter of concern, but the evaluation times chosen, particularly 15 and 30 minutes after reperfusion, provided comparable washout for the two techniques.

The correlation between the extent of free radical production and the duration of ischaemia has been investigated in vivo and in vitro experimental postischaemia models^{8 45 46} and more recently in postcardioplegic arrest in humans hearts.²⁰ In the present study there was no statistically significant correlation between the duration of ischaemia and the appearance of biological signs of oxidative stress in either group. This can be taken as additional proof of the antioxidant efficacy of blood in the cardioplegic solution^{19 44} especially for the CBC group. Blood appears attractive from the standpoint of inherent mechanisms for prevention of free radical generation: in addition to the antioxidants contained in plasma (urate, plasma proteins, ascorbate, vitamin E), erythrocytes contain catalase, superoxide dismutase, and glutathione all of which participate in reactions to scavenge free radicals. Indeed, no major efflux of GSH (mainly related to unspecified membrane damage) from reperfused hearts was detected in either cardioplegia group in contrast with other reports where crystalloid cardioplegia was employed.¹⁷ The demonstration that erythrocyte glutathione is oxidised to GSSG proves that intra-erythrocyte antioxidant defences are actively engaged during reperfusion of the CBC group.

The role of hypothermia as a protective agent during cardioplegic arrest is controversial; hypothermia seems to alter tissue oxygen delivery and reduce the cell's ability to produce ATP efficiently after a period of anaerobic arrest.^{22 46} Furthermore, it is widely known that the activities of many enzymes involved in crucial metabolic pathways or free radical detoxification are inhibited by low tissue temperature.³¹

The most meaningful finding in our series was the lack of correlation between oxidative stress and immediate postoperative ventricular dysfunction; indeed there was no significant difference between groups in common haemodynamic parameters (table 2 and fig 4) or need of pharmacological or mechanical support. Even if the differences of DO_2I and VCO_2I three hours after ICU admission (T3) could be due to the lower metabolic debt, lower peripheral vasoconstriction and better tissue perfusion of the WBC compared with the CBC group, the latter also showed improved and uneventful post-surgical courses.

We could perhaps concur with Ki-Bong⁴⁷ that oxygen free radicals play an important role in in vivo myocardial reperfusion stress, but endogenous self-defensive antioxidative enzyme systems are also triggered leading to

significant myocardial cellular damage. Indeed the complete reduction of that oxidative stress does not affect the postoperative recovery of myocardial function in a low risk carefully selected group of patients. Additional studies are required to assess the contribution of free radicals to the clinical pathology associated with surgical procedures in high risk patients where the presence of postoperative myocardial stunning can significantly affect mortality and morbidity.

The work has been supported in part by MURST 60% grant. We thank Miss Laura Berto for preparation of the graphics and manuscript.

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